Cultivation of Viruses

Botany
BBZ

- Viruses do not fall in the category of unicellular microorganism
- They are obligate intracellular parasites and lack the machinery necessary for protein and nucleic acid synthesis
- They depend on the host machinery for their growth and survival
- Unlike other microorganism, complex processes are involved in their multiplication
- Outside of the host cells, viruses are inactive, however, inside living cells, viruses show some of the characteristics of living things

Cultivation of Virus

- Since the viruses are obligate intracellular parasites, they cannot be grown on any inanimate culture medium
- Viruses can be cultivated within suitable hosts, such as a living cell
- The primary purposes of viral cultivation are:
  1. To isolate and identify viruses in clinical specimens
  2. To prepare viruses for vaccines
  3. And to do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells

Cultivation of Virus

- Viruses not only need living cells to grow in but also they are specific about the type of cell they infect and grow in
- There is no universal cell that will support all viruses
- Viruses tend to be host specific; therefore:
  - human viruses grow best in cells of human origin,
  - bovine viruses in bovine cells,
  - canine viruses in canine cells,
  - while some viruses will not grow in vitro at all
- Therefore in the laboratory the suspected virus must be grown in a culture method known to support its growth

Methods for Cultivation of Virus

- Animals are used for studying viruses which do not grow in cell cultures or eggs, and for testing vaccines
- Eggs support a fairly wide range of animal and human viruses – hence their importance in the diagnostic service
- Cell cultures; different types of cell lines will support different types of viruses

Inoculation of Virus in Animals

- Laboratory animals play an essential role in studies of viral pathogenesis
- Live animals such as monkeys, mice, rabbits, guinea pigs, ferrets are widely used for cultivating virus
- Mice are the most widely employed animals in virology

Inoculation of Virus in Animals

- The different routes of inoculation in mice are:
  - intracerebral
  - subcutaneous
  - intraperitoneal
  - or intranasal
- After the animal is inoculated with the virus suspension, the animal is:
  - observed for signs of disease
  - visible lesions
  - or is killed so that infected tissues can be examined for virus

Cell Cultures

- Prior to the advent of cell culture, animal viruses could be propagated only on whole animals or embryonated chicken eggs
- Cell cultures have replaced embryonated eggs as the preferred type of growth medium for many viruses
- Cell culture consists of cells grown in culture media in the laboratory
- These cultures can be propagated and handled like bacterial cultures; they are more convenient to work with than whole animals or embryonated eggs

Inoculation of Virus in Embryonated Eggs

- Goodpasture and Burnet in 1931 first used the embryonated hen's egg for the cultivation of virus
- The process of cultivation of viruses in embryonated eggs depends on the type of egg being used
- Eggs provide a suitable means for:
  - the primary isolation and identification of viruses
  - the maintenance of stock cultures
  - and the production of vaccines

Embryonated Egg

- Terms most often refer to eggs:
  - Embryonated: having an embryo
  - Unembryonated: not having an embryo
  - De-embryonated: having lost an embryo
- Embryonated egg, referring to an advanced stage of development and not merely after fertilisation

Advantages

- An embryo is an early developmental stage of animals marked by rapid differentiation of cells
- Birds undergo their embryonic period within the closed protective case of an egg, which makes an incubating bird egg a nearly perfect system for viral propagation
- It is an intact and self-supporting unit, complete with its own sterile environment and nourishment
- It furnishes several embryonic tissues that readily support viral multiplication
- Defense mechanisms are not involved in embryonated eggs
- Cost- much less, Maintenance-easier, Less labor and Readily available

Inoculation of Virus

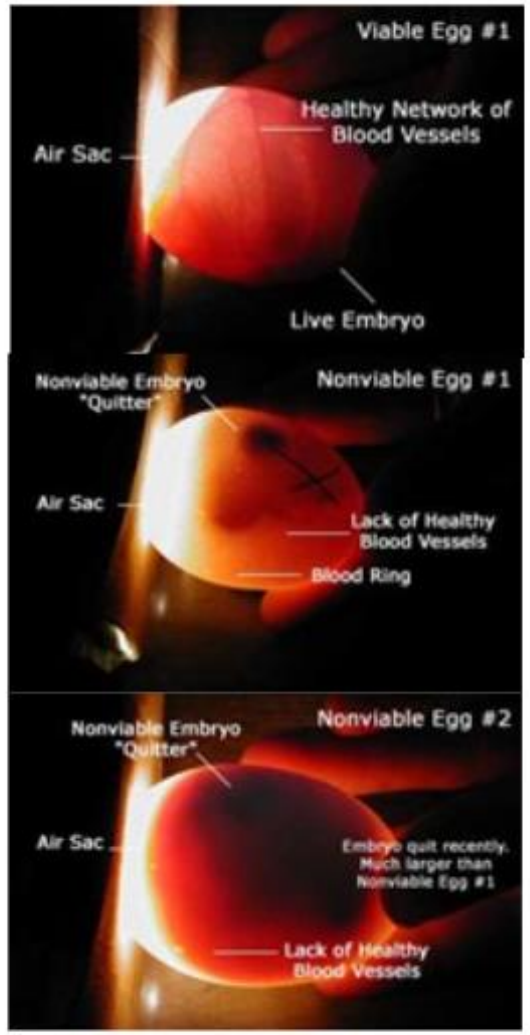
- Chicken, duck, and turkey eggs are the most common choices for inoculation
- The egg used for cultivation must be sterile and the shell should be intact and healthy
- Rigorous sterile techniques must be used to prevent contamination by bacteria and fungi from the air and the outer surface of the shell

Inoculation of Virus

- The egg must be injected through the shell, usually by drilling a hole or making a small window
- The viral suspension or suspected virus- containing fluid is injected into the fluid of the egg
- The exact tissue that is inoculated is guided by the type of virus being cultivated and the goals of the experiment

Detection of Viral Growth

- Viruses multiplying in embryos may or may not cause effects visible to the naked eye
- The signs of viral growth include:
  - Death of the embryo
  - Defects in embryonic development
  - and localized areas of damage in the membranes, resulting in discrete opaque spots called pocks

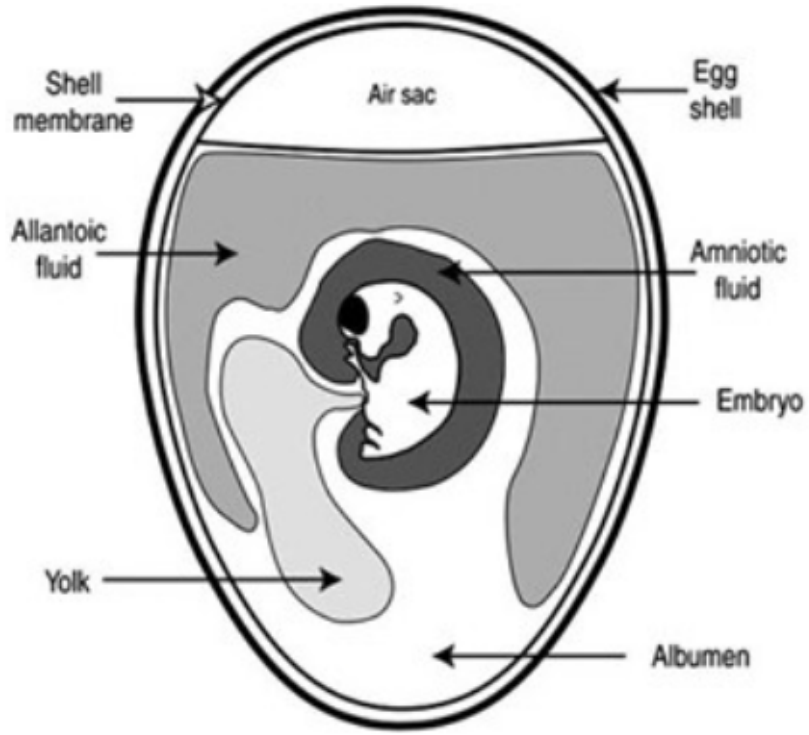


Detection of Viral Growth

- If a virus does not produce obvious changes in the developing embryonic tissue, virologists have other methods of detection
- Embryonic fluids and tissues can be prepared for direct examination with an electron microscope
- Certain viruses can also be detected by:
  - their ability to agglutinate red blood cells
  - or by their reaction with an antibody of known specificity

Parts of Embryonated Egg

- The air sac is important to the developing embryo for respiration and for pressure adjustments
- The shell and shell membrane function both as a barrier and as an exchange system for gases and liquid molecules
- The chorioallantoic sac and its contents (allantoic fluid) remove waste products produced by the developing embryo
  - This Membrane and its contents increases in size as the embryo grows

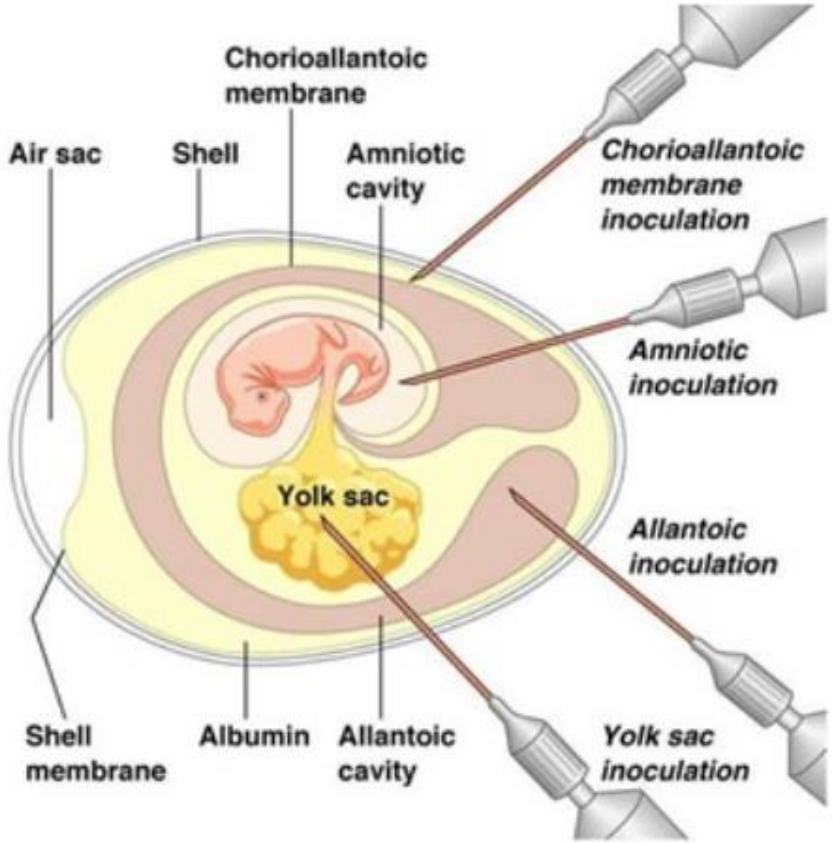


Parts of Embryonated Egg

- The yolk sac is the source of nourishment for the developing Embryo
  - As the embryo develops, the yolk sac decreases in size until it is completely absorbed into the digestive system of the mature embryo
- The amnion is a thin membrane that encloses the embryo and Protects it from physical damage
  - It also serves as an exchange system and is best seen in the younger embryos

Routes of Viral Inoculation

- An embryonated egg offers various sites for the cultivation of viruses
- The different sites of viral inoculation in embryonated eggs are:
    1. Chorioallantoic membrane(CAM)
    2. Amniotic Cavity
    3. Allantoic Cavity
    4. Yolk sac



Routes of Viral Inoculation

- The chosen route of inoculation and age of the embryo are determined by the given virus selectivity for a certain membrane or developmental stage of the embryo
  - For example Infectious bronchitis virus is propagated in the yolk sac of a 5-6 day old embryo
  - whereas Rous-sarcoma virus is inoculated on the chorioallantoic membrane of a 9-11 day old embryo and will produce pocks 5-10 days post-infection

Candling of Egg

- Candling is the process of holding a strong light above or below the egg to observe the embryo
- A candling lamp consists of a strong electric bulb covered by a plastic or aluminum container that has a handle and an aperture